 0030-0950/80/0005-0206 $2.00/0

PESTICIDE ANALYSIS, EGG AND EGGSHELL CHARACTERISTICS OF RED-TAILED HAWK EGGS[1]

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Abstract. In this study, addled and viable Red-tailed Hawk eggs were compared in terms of pesticide levels, egg and eggshell parameters, and shell ultrastructure as determined by scanning electron microscopy. These values were then used to compare actual measurements against egg and eggshell indices commonly reported in the literature. Addled eggs of Red-tailed Hawks were of greater length, weight and volume than viable eggs, while viable eggs showed greater shell weights and thickness than addled eggs. The general structure of the eggshell consisted of 3 layers: shell membrane, mammillary layer and palisade layer. No cuticle was present. No differences in thickness of the shell membrane or mammillary layer were detected between addled and viable eggs; however, the palisade layer was found to be 24% thicker (X_2, $P<0.05$) in viable eggs than in addled eggs (0.11 ± 0.03 mm and 0.08 ± 0.01 mm, respectively). The thicker palisade layer resulted in an overall thicker eggshell in viable compared to addled eggs (0.28 ± 0.02 mm and 0.25 ± 0.03 mm, respectively). Extensive areas of cavitation in the mammillary layer were found in many addled eggs, but never in viable eggs. Pore channels originating at the membrane layer could be seen extending to the outer surface of the egg. All egg samples analyzed for pesticide residues bore measureable amounts of PCB and DDE, while some samples contained dieldrin and heptochlor epoxide.

OHIO J. SCI. 80(5): 206, 1980

During the past twenty years, numerous studies have reported residues of organochlorine insecticides in the eggs of wild birds, but few studies have concentrated on buteos. Seidensticker and Reynolds (1971) analyzed eggs of Red-tailed Hawks in Montana and found measurable, but relatively low, levels of DDT, DDD, DDE, and dieldrin in all eggs tested. Heptochlor epoxide and PCB was found in some samples, and low levels of DDE were reported in Canadian Red-tailed Hawks (Postupalsky 1970). In both falconiforme and non-falconiforme birds, changes in parameters such as volume, size and shape have been related to geographical location and/or natural variation in egg sizes within clutches or species (Osborne and Winters 1977). Eggshell weight (Ratcliffe 1967) and eggshell thickness have been shown to be inversely proportional to residue levels in the egg (Anderson and Hickey 1970). Through the use of scanning electron microscopy (SEM), structural changes in the eggshell have been shown to correlate with high pesticide levels in the egg of non-falconiforme birds (McFarland *et al* 1971, Jorgenson and Kraul 1974).

The purpose of the present study was to compare addled and viable eggs of Red-tailed Hawks in terms of pesticide levels, egg and eggshell parameters, and shell ultrastructure. The values were then used to compare actual measurements against egg and eggshell indices commonly reported in the literature.

MATERIALS AND METHODS

Seventy addled Red-tailed Hawk eggs representing 63 clutches were randomly collected in 6 Ohio counties (Butler, Delaware, Franklin, Hamilton, Preble and Warren) and 3 counties in Kentucky (Boone, Pendleton and Timble) during 1967–77. Eight viable eggs representing 6 clutches were collected in 2 Ohio counties (Delaware and Butler) in 1978. Addled eggs were collected in Ohio during raptor censusing by Jack Holt and myself while addled eggs from Kentucky were collected by Jack Holt. After

[1]Manuscript received 12 March 1979 and in revised form 5 October 1979 (#79-15).

weights and linear measurements were taken, the eggs were frozen and stored at −5 °C for subsequent chemical analysis. Fertilization was determined by candling.

Egg and Eggshell Parameters. Fresh eggs were weighed to the nearest 0.01 g and compared to the weight calculated using the index of Romanoff and Romanoff (1949). Egg volume was measured to the nearest ml by water displacement and compared to the estimate obtained using the index of Kendeigh *et al* (1956). These index values allow one to compare this study's values with those reported by Osborne and Winters (1977).

A Starrett 1010 M dial micrometer was used to measure eggshell thickness (minus membranes). The average of 3 measurements taken at the equator of the egg was used as an index of shell thickness and compared with the thickness index as defined by Ratcliffe (1967, 1970), which was also used to determine relative eggshell thickness. This index has been used to document the relationship between eggshell thinning and the increase of persistent pesticides in the environment (Anderson and Hickey 1970, Cooke 1973). Ultrastructure of eggshells from 25 viable and 25 addled eggs randomly picked was examined with scanning electron microscopy (SEM). Eggs were radially-fractured, mounted on a stub, coated with gold paladium and examined with a Coates and Welter SEM.

Twelve addled and 3 viable eggs were randomly picked for pesticide analysis conducted by the Ohio Department of Agriculture, Reynoldsburg, Ohio. Sample size was limited by financial considerations and technical assistance. Badly decomposed or dehydrated eggs were eliminated from the study. The contents of the eggs were prepared for chemical analysis by homogenization, divided into 25 g samples and analyzed by standard gas chromatographic techniques. The organochlorine pesticides and polychlorinated biphenyls (PCB's) were separated in two 6 ft by 2 mm ID glass columns and analyzed by a Hewlett Packard linear electron capture gas chromatograph. Column packing material in column number one was 4% OV101 chromsorb W HP with 100–120 meshing. In column number two, a 1:1 ratio of 4% OV101 chromsorb W HP and 6% OV210 chromosorb W HP with 100–120 meshing was used. Eggs were analyzed for DDT, metabolites (DDE), dieldrin, heptochlor epoxide (Heptochlor), PCB AR 1254/60 (PCB's). Recovery ranged from 80 to 100% with an average recovery of better than 90%. A more detailed description of the analytical procedure is presented by Riechel (1977).

Data was statistically analyzed with anova, student's t-test, and chi-square tests. Since no statistical differences were found due to geographical variation, single egg samples per clutch versus multiple egg samples per clutch or between different years, egg values were pooled for statistical analysis.

RESULTS AND DISCUSSION

Egg and Eggshell Parameters.

Fresh egg weights of the viable eggs were 5% lower than the estimated egg weights calculated by the Romanoff weight index. The actual egg weights of addled eggs were not taken inasmuch as this measurement is considered an unreliable index because of dehydration, decomposition, etc. of addled eggs. As determined by the weight index (Romanoff and Romanoff 1959), egg weights ranged between 7% to 9% higher in addled eggs than in viable eggs ($P<0.05$). Less than 2% variation was found in the weight index of addled eggs.

Addled eggs ranged between 3% to 5% longer than viable eggs ($P<0.05$) while breadth of addled eggs was only 2% larger than viable eggs. Variations in length and breadth between different years in addled eggs was 4% and 2%, respectively (see table 1).

Mean volume (actually measured by water displacement) of addled eggs was

TABLE 1

Egg and Eggshell Characteristics of Red-tailed Hawk Eggs.

Year	Egg Condition	No. of Eggs	Length (mm)	Breadth (mm)	Egg Weight Index*	Volume (cm^3)	Volume Index**	Shell Weight (gm)	Shell Thickness (mm)	Thickness Index†
1974	Addled	13	59.4±2	46.8±1	72.4±5	67.2±4	68.1±5	5.7±.4	.359±.03	2.24±.1
1975	Addled	8	59.3±3	46.2±1	71.0±4	65.7±4	66.4±5	6.0±.3	.348±.02	2.36±.2
1976	Addled	18	60.5±3	47.4±2	75.9±6	70.1±6	71.0±6	5.9±.5	.348±.02	2.25±.2
1977	Addled	8	60.4±2	47.3±2	75.9±7	69.4±6	69.4±6	6.2±.3	.363±.02	2.36±.2
	$\bar{X}$ (n=47)		59.9±2	47.0±2	73.8±6	68.1±5	68.7±5	5.9±.4	.355±.03	2.26±.1
1978	Viable	8	57.4±2	46.4±1	69.2±5††	60.5±4	64.2±4	6.3±.3	.420±.03	2.36±.2
(Pooled)	$\bar{X}$ (n=55)		59.4±2	46.9±1	72.8±5	66.56±5	67.8±5	6.0±.3	.367±.03	2.29±.1

*Weight Index (Romanoff and Romanoff 1949).
**Volume Index after Kendeight *et al* (1956).
†Thickness Index (Ratcliffe 1967).
††Actual mean fresh egg weight of viable eggs. 65.7±3.5

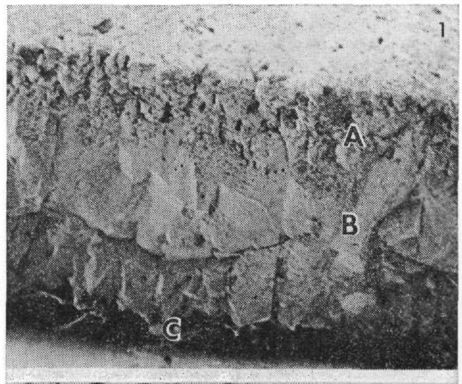
4% to 10% higher than viable eggs ($P<.01$). Variation in actual volume of addled eggs was 6%. Volume, as determined by the index of Kendeigh, averaged 2% higher than actual volumes, whereas volume based on fresh egg weights (Pagnelli *et al* 1974) underestimated the actual volume by 1%. These differences in egg volume could be attributed to biases in the formulas but may also entail natural variations in egg size within the clutch or species (Osborne and Winter 1977).

Shell weight of addled eggs was 4% to 9% less than that of viable eggs. Shell weight, as determined by the formula of Pagnelli *et al* (1974), underestimated the actual shell weight by 10% as compared to my actual weights.

Shell thickness of addled eggs was 13% to 17% less than that of viable eggs ($P<0.05$) and compared to the thickness index of Ratcliffe, ranging from 1% to 6% lower than the index of viable eggs. Thickness index values of viable eggs averaged 2% higher than Anderson and Hickey's California eggs, but 2% lower than Ohio eggs as reported by Osborne and Winters (1977). These values were 5% higher than in red-tailed hawk eggs from California reported by Anderson and Hickey (1970b). These differences in thickness index could be attributed to natural variations in the egg size within the clutch or species.

Eggshell Structure.

The general structure of the Red-tailed Hawk eggshell as viewed with a SEM resembled that previously reported for falconiformes (Tyler 1969). The eggshell consisted of 3 layers: shell membrane, mammillary layer, and palisade layer (figure 1). No cuticle layer was present. The shell membrane contained an inner and an outer layer, the inner layer being relatively smooth with irregularly spaced pores and the outer layer appearing as a rough, intercalating network of fibers. The mammillary layer also consisted of 2 distinct layers, *i.e.*, an upper and a lower layer. These layers were usually separated by horizontal plates whose composition appeared similar to that of the remaining mammillary layer and whose thickness ranged from 1 to 2 mm (figure 2). When these plates were absent, a distinct line between the two lamellae was present. Tyler (1969) did not report the separation of the mammilary layer. The membrane surface of the bottom layer had the characteristic knobs to which the shell membranes attached (Tyler 1966). The palisade layer covered the surface of the eggshell and was characterized by small irregularly-spaced pores. Contrary to Tyler (1969), pore channels originating at the membrane layer could be seen extending to the outer surface of the egg (figure 3). Areas of pigmentation were found in the palisade layer with the structure of pigmentation varying from reticular to globular and thickness from



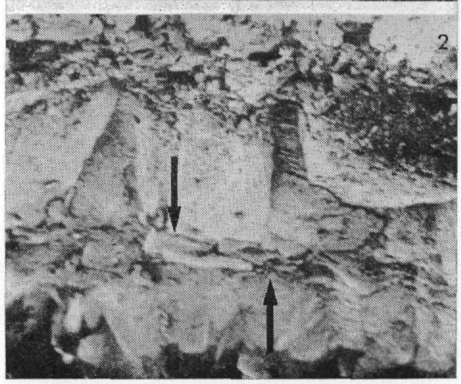


FIGURE 1. SEM photomicrography of a viable Red-tailed Hawk eggshell: A = Palisade Layer, B = Mammillary Layer, C = Shell Membrane.

FIGURE 2. SEM photomicrograph of a viable Red-tailed Hawk eggshell showing horizontal plates in mammillary layer (see arrows).

1 to 4 μ. Pigmentation was never seen penetrating farther than the palisade layer.

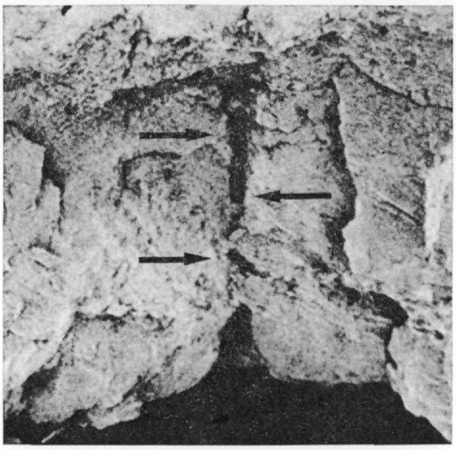


FIGURE 3. SEM photomicrograph of a viable Red-tailed Hawk eggshell showing pore channels (see arrows).

No differences in thickness of the shell membrane or mammillary layer were detected between addled and viable eggs. The palisade layer, however, was found to be 24% thicker ($P<0.05$) in viable than in addled eggs and measured 0.11 ± 0.03 mm and 0.08 ± 0.01 mm, respectively. The thicker palisade layer resulted in an overall thicker eggshell in viable compared to addled eggs (0.28 ± 0.03 and 0.25 ± 0.02 mm). Extensive areas of cavitation in the mamillary layer were found in many addled eggs but never in viable eggs (figure 4).

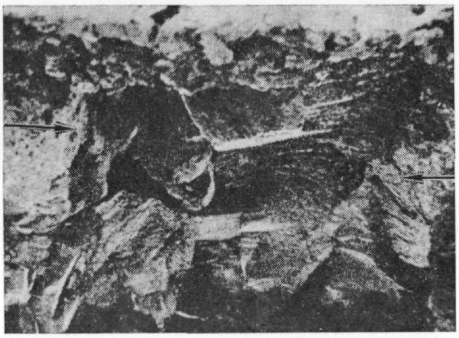


FIGURE 4. SEM photomicrograph of an addled Red-tailed Hawk eggshell showing extensive cavitation in mammillary layer (see arrows).

Pesticide Analysis.

Egg samples analyzed for pesticide residues contained measurable amounts of PCB and DDE; 13 of 15 samples contained dieldrin, and 12 of 15 samples contained Heptochlor. Of the 15 samples analyzed, PCB was the most abundant residue in 13, whereas dieldrin was the most abundant residue in 2 samples (table 2).

TABLE 2

Pesticide Analysis of Red-tailed Hawk Eggs. A Comparison Between Springer 1979 (S) and Seidensticker and Reynolds 1971 (SR).

	DDE (ppm)		Dieldrin* (ppm)		Heptochlor (ppm)		PCB (ppm)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Addled								
S 1976 (n=7)	0.71±0.9	0.14–2.9	1.27±1.4	0–3.6**	0.23±0.1	0.03–0.1	3.71±5.1	0.62–16.1
S 1977 (n=5)	0.62±0.9	0.15–1.9	0.88±0.5	0.41–1.3	0.11±0.1	0.06–0.2	1.03±0.6	0.35–1.7
SR 1971 (n=2)	4.19±	1.08–10.3	0.39±	0.23–0.6	0.51±	0.3–0.8	—	—
Viable								
S 1978 (n=3)	0.18±0.1	0.13–0.3	0.53±0.6	0.12–1.2	—	—	0.40±0.2	0.22–0.7
SR 1971 (n=3)	0.92±	0.24–1.6	0.30±	0.16–0.4	0.16±	0.09–0.2	0.25±**	—

*Since Aldrin is rapidly converted to dieldrin, the same figures will apply to these compounds (Prestt and Ratcliffe 1970). All residues were measured as ppm net weight.
**Based on one sample.

In comparing our pesticide data with those reported by Seidensticker and Reynolds (1971), there were differences in levels of DDD, DDE, dieldrin and PCB between the two studies. Whereas Seidensticker and Reynolds (1971) reported high concentration of DDD and low levels of dieldrin and PCB, we found small quantities of DDE, no DDT or DDD, and high levels of dieldrin and PCB. Since aldrin rapidly converts to dieldrin, our high concentration of dieldrin probably reflects the heavy use of aldrin on Ohio cornfields in the past. Levels of this magnitude in the Scottish Golden Eagles were associated with severe problems such as increased hatchability (Lockie *et al* 1969). The average PCB level for addled eggs for this study was skewed relatively high by one sample containing 16.1 ppm, but without this sample, PCB's averaged 1.55 ppm for the 1976–1977 sampling years. This average represents a seven-fold increase in concentration over that reported in viable eggs by Seidensticker and Reynolds (1971). In my study over a 3 year period, residues of DDT and metabolites dieldrin and PCB decreased, while Heptochlor levels remained relatively constant.

Addled eggs had significantly higher pesticide levels ($P<0.05$) than viable eggs in both years. Since eggshell thickness is inversely proportional to egg pesticide residue levels (Anderson and Hickey 1970) and ultrastructural changes in mammillary layers are directly proportional to pesticide residues in the egg (Jorgenson and Kraul 1974), the high pesticide levels observed in my study may have resulted in the structural differences found between addled and viable eggs.

Acknowledgments. Special thanks to Jack Holt for supplying some of the addled eggs, to the Ohio Division of Wildlife and Ohio Department of Agriculture for financial assistance, to Dr. David Osborne for his advice throughout the study, to Dr. David Garvey for his manuscript reviewal and to Penny Hedberg, Rebecca Edgerton and Jane Cramer for their secretarial assistance on this manuscript. This manuscript was partially derived from a M.S. thesis project at Miami University, Oxford, Ohio.

LITERATURE CITED

Anderson, D. W. and J. J. Hickey 1970 Eggshell changes in certain North American birds. pp. 514–540. Symposium on Man and Birds: Proc. 15th Inter. Ornith. Cong., Hague.

——— and ——— 1970b Oological data on egg and breeding characteristics of Brown Pelicans. Wilson Bull. 82: 14–28.

Cooke, A. S. 1973 Shell thinning in avian eggs by environmental pollutants. Environ. Pollut. 4: 85–152.

Jorgenson, O. H. and I. Kraul 1974 Eggshell parameters and residues of PCB and DDE in eggs from Danish Herring Gulls *Larus a. argentatus*. Ornis. Scand. 5: 173–179.

Kendeigh, S. C., T. C. Kramer and F. Hammerstrom 1956 Variations in egg characteristics of the House Wren. Auk 73: 42–64.

Lockie, J. D., R. A. Ratcliffe and R. Balharry 1969 Breeding success and organochlorine residues in Golden Eagles in West Scotland. J. Appl. Ecol. 6: 381–389.

McFarland, L. Z., R. L. Garrett and J. A. Nowell 1971 Normal eggshells and thin eggshells caused by organochlorine insecticides viewed by the Scanning Electron Microscope. SEM Symposium IIT. 4: 377–384.

Osborne, D. R. and R. Winters 1977 Pre-1941 eggshell characteristics of some birds. Ohio J. Sci. 77: 10–23.

Pagnelli, E. V., A. Olfzowka and A. Az 1974 The avian egg: Surface area, volume and density. Condor 43: 216–218.

Postupalsky, S. 1970 Effects of pesticides on populations of Bald Eagle and other fish-eating birds in the Lake Nipigon and Lake Superior region of Ontario: Report submitted to the Canadian Wildlife Service, Ottawa, Canda. 29 pp.

Prestt, I. and D. A. Ratcliffe 1970 Effects of organochlorine insecticides on European birdlife. pp. 486–513. Symposium on Man and Birds: Proc. 15th Inter. Ornith. Cong., Hague.

Ratcliffe, D. A. 1967 Decrease in eggshell weight in certain birds of prey. Nature 215: 208–210.

——— 1970 Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds. J. Appl. Ecol. 7: 67–113.

Reichel, W. L. 1977 Putuxent Analytical Manual, Environmental Residues Chemistry Project, U. S. Fish and Wildlife Service, Putuxent Wildlife Research Center, Laurel, Maryland 20811.

Romanoff, A. L. and A. J. Romanoff 1949 The Avian Egg. Wiley and Sons, New York. 918 p.

Seidensticker, J. C. and H. V. Reynolds 1971 The nesting, reproductive performance, and chlorinated hydrocarbon residues in the Red-tailed Hawk and Great Horned Owl in south-central Montana. Wilson Bull. 83: 408–418.

Tyler, C. 1969 Avian eggshells: their structure and characteristics. Gen. Exp. Zool. 4: 81–131.

——— 1966 A study of the eggshells of the falconiformes. Zool. J. 150: 413–425.